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DAC  
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Practitioner's Docket No. U 013488-3

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Pushpa KHANNA

Serial No.: 09/881,569

Group No.: 1653

Filed: June 14, 2001

Examiner.: Sheridan Snedden

Confirmation No.: 5858

For: PROTEIN/POLYPEPTIDE-K OBTAINED FROM MOMORDICA  
CHARANTIA AND A PROCESS FOR THE EXTRACTION THEREOF

Mail Stop Petition

Commissioner for Patents

P.O. Box 1450, Alexandria, VA 22313-1450

PETITION FOR LATE ENTRY OF PRIORITY CLAIM  
AND/OR PRIORITY PAPERS UNDER 37 CFR § 1.55(A)

PETITION

1. Applicant petitions for entry of the following accompanying papers with respect to the priority claim in this case being made after payment of the issue fee on May 12, 2004.

**CERTIFICATION UNDER 37 C.F.R. 1.8(a) and 1.10\***

*(When using Express Mail, the Express Mail label number is mandatory;  
Express Mail certification is optional.)*

I hereby certify that, on the date shown below, this correspondence is being:

**MAILING**

- ☒ deposited with the United States Postal Service in an envelope addressed to the Commissioner for Patents, P. O. Box 1450, Alexandria, VA 22313-1450.

**37 C.F.R. 1.8(a)**

- ☒ with sufficient postage as first class mail.

**TRANSMISSION**

- ☐ transmitted by facsimile to the Patent and Trademark Office.

Date: May 19, 2004

**37 C.F.R. 1.10\***

☐ as "Express Mail Post Office to Address"  
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Signature

**CLIFFORD J. MASS**

*(type or print name of person certifying)*

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"Since the filing of correspondence under § 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will **not** be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.



☒ Certified copies of the application from which priority is claimed:

Country: India  
Application No.: 561/Del/99  
Filing Date: April 13, 1999

Country: India  
Application No.: 560/Del/99  
Filed: April 13, 1999

Fee

The petition fee (37 CFR § 1.17(i)) of \$130.00 is to be paid as follows:

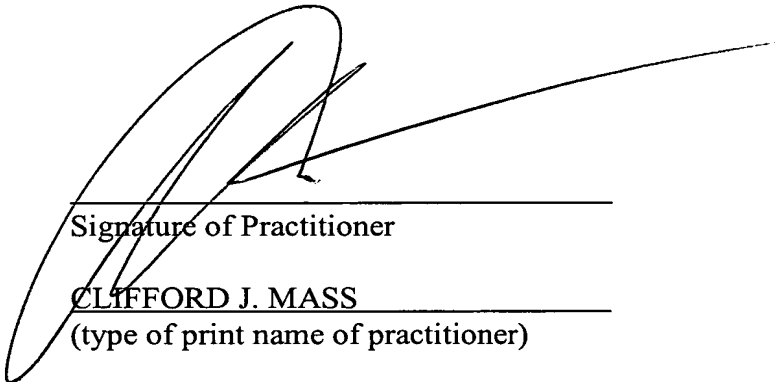
Attached is a ☒ check ☐ money order in the amount of \$130.00

☒ Charge any additional fees required by this paper or credit any overpayment to  
Account No. 12-0425.

Reg. No.: 30,086

Tel. No.: (212) 708-1890

Customer No.: 00140



\_\_\_\_\_  
Signature of Practitioner

CLIFFORD J. MASS  
\_\_\_\_\_  
(type of print name of practitioner)

\_\_\_\_\_  
P.O. Address

\_\_\_\_\_  
LADAS & PARRY  
26 WEST 61ST STREET  
NEW YORK, NEW YORK 10023





सत्यमेव जयते



INTELLECTUAL  
PROPERTY INDIA

GOVERNMENT OF INDIA  
MINISTRY OF COMMERCE & INDUSTRY,  
PATENT OFFICE, DELHI BRANCH,  
W - 5, WEST PATEL NAGAR,  
NEW DELHI - 110 008.

*I, the undersigned, being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the Application and Complete Specification and Drawing Sheets filed in connection with Application for Patent No.560/Del/99 dated 13<sup>th</sup> April 1999.*

*Witness my hand this 3<sup>rd</sup> Day of February 2003.*

**(H.C. BAKSHI)**

*Joint Controller of Patents & Designs.*



11-4-99

18 JAN 2002

## FORM I

## THE PATENT ACT, 1970

APPLICATION FOR PATENT WHEN THE TRUE AND FIRST INVENTORS  
ARE THE SOLE JOINT APPLICANTS (SEE SECTION)

I, PUSHPA KHANNA, E-1417, VASANT VIHAR, NEW DELHI - 110057. INDIAN  
CITIZEN, hereby declare :-

- (i) that I, Pushpa Khanna is in possession of an invention relates to an improved process for preparation of highly effective hypoglycaemic polypeptide-k from a ~~plant source~~, seeds of Momordica Charantia
- (ii) that I, Pushpa Khanna claims to be true and first inventor therefore;
- (iii) that the specifications filled with this application is complete and any amended specification which may hereafter be filed in the behalf will be, true of the invention to which this application relates,
- (iv) that I, Pushpa Khanna believe that I, Pushpa Khanna is entitled to a patent for the said invention having regard to the provision of the Patent Act, 1970;
- (v) that to the best of my knowledge, information and belief the facts and matters stated herein are correct and there is no lawful ground of objection to the grant of patent to me on this application.

I, Pushpa Khanna request that a patent may be granted to me for the said invention and I, Pushpa Khanna request that all notices, requisitions and communications relating to this application may be sent to PUSHPA KHANNA, E-1417, VASANT VIHAR, NEW DELHI - 11 0057.

Dated : this 17<sup>th</sup> day of January 2002

To,

The Controller  
The Patent Office  
5, West Patel Nagar  
New Delhi

Pushpa Khanna  
(Signed) - I. Pushpa Khanna

18 JAN 2002

ORIGINAL



0530 DEL 99

FORM 3A

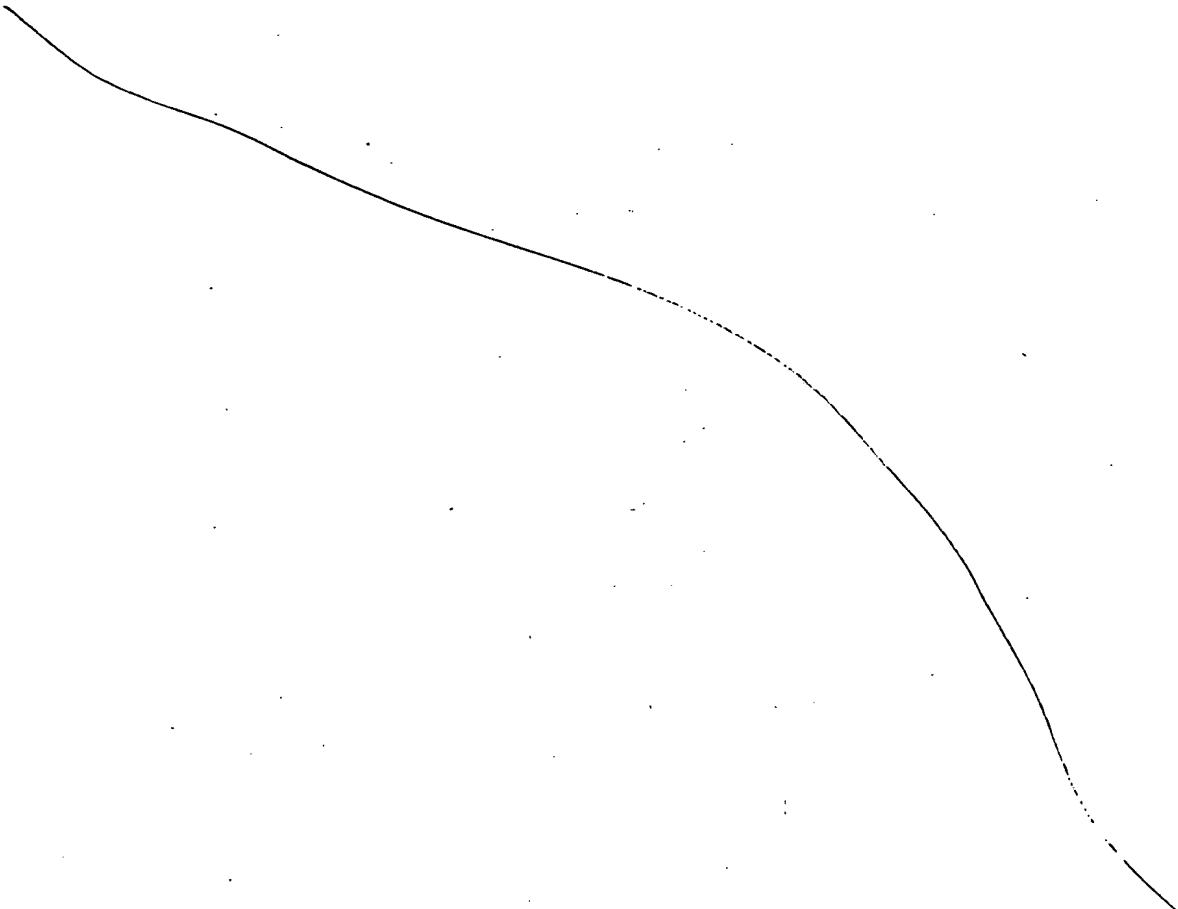
21999

THE PATENTS ACT, 1970

Complete Specifications  
(See Section 10)

1. AN INVENTION RELATES TO AN IMPROVED PROCESS FOR PREPARATION OF HIGHLY EFFECTIVE HYPOGLYCAEMIC POLYPEPTIDE-~~R~~ FROM A PLANT SOURCE.
2. PROF. (DR.) MISS PUSHPA KHANNA (RETD.) E-14/7, VASANT VIHAR, NEW DELHI - 110057. INDIAN CITIZEN

The following specifications particularly described and ascertains the nature of this invention and the manner in which it is to be performed:-



This invention relates to an improved process for preparation of highly effective hypoglycaemic polypeptide-k from seeds. This invention particularly relates to process for preparation of sublingually highly effective hypoglycaemic polypeptide-k extracted from seeds of Momordica Charantia L.

Insulin has hitherto been commercially or synthesised from the pancreas of animals and human insulin from E. Coil (Eli-Lily U.S.A.). So far there is no commercial extraction of insulin / polypeptide-k from plant source.

Isolation of Insulin from animal pancreas is open to objection due to the following reasons :

1. By killing 10,000 animals only one pound of pure insulin is obtained.
2. It is not being sublingually administered.
3. If the pancreas are infected by some diseases like Cancer etc. there is always a probability of its being carried (if it is a virus) along with the insulin.
4. Human Insulin can be synthesised from E. Coil, which is expensive.
5. Any amount of polypeptide-k (p) can be synthesised from plant which is so easy to cultivate.

*Prashant Khanna*

An attempt was made to extract polypeptide-k from seeds of Momordica Charantia L. by the present applicant. The method used was discussed in old Indian Patent Specification No. 136565 which removed the above mentioned drawbacks. The fruit and cultures are separately extracted in ethanol and then mixed with cold ethanol and diethyl ether, needle like crystals formed by adding zinc in traces after 18 hrs. The fruit and cultures are separately crushed, homogenized in water, ethanol and concentrated sulfuric acid added adjusting pH 1.5-2.0, filtered.

This method had the following drawbacks :

1. The use of alcohol in the extraction procedure was not practical due to its unavailability in large amount and the impurities present in it.
2. The use of raw material as fruits and tissue culture creates problems in handling the uneconomic viability respecting and the yield was very poor.

In order to obviate the above drawbacks another process was developed for extraction of a highly effective polypeptide-~~R~~ by using hexane alongwith diethylether which was claimed in Indian patent specification No. 176040. Although the process developed and disclosed in above referred patent specification resulted into good yield, improved purity and high efficacy of the drug by removal of oil and sapogenins and other contaminants therefrom. Yet it had some drawbacks :

1. The purification of polypeptide-p was cumbersome method due to the presence of interfering radicals as oil and sapogenins.
2. Use of diethylether in the extraction procedure was not practical due to its being highly inflammable and involving high cost.
3. The presence of pesticides / insecticides / urea and other contaminants affected the purity of polypeptide-~~R~~ - ~~R~~
4. The yield was not optimum.

Now I have developed an improved process and a change in raw material as seeds for extracting a highly effective hypoglycaemic polypeptide from seeds and obviated drawbacks. In this process, splitted seeds were, thoroughly washed with water many times to remove the last traces of pesticides / insecticides / urea and other contaminants.

The dried seeds are processed using hexane (food grade) along with acetone instead of diethyl ether used in the process described in earlier Patent No. 176040. The process has resulted into high yield, improved purity and high efficacy of drug by removal of undesired oils, flavonoids and sapogenins therefrom. The doses of resulted drug were administered sublingually to diabetic patients and showed excellent results in lowering the blood sugar level.

Accordingly the present invention provides an Improved process for preparation of a sublingually highly effective hypoglycaemic novel protein (Polypeptide-K) from seeds of Momordica Charantia L. (bitter gourd) which comprises splitting the seeds of said Momordica Charantia, washing thoroughly the splitted seeds with water to remove contaminants, drying, powdering and treating the powder with hexane and acetone (2:1) to remove oil, drying the residual mass and dissolving the same in water and acetone, adjusting the pH to 9.5 by adding ammonium hydroxide/organic buffer, separating the supernatant layer from the mixture, treating the supernatant layer with sulfuric acid to adjust the pH to 3 to obtain the flocculent precipitate of polypeptide-k hydrolyzate, and crystallizing the said polypeptide -k with zinc acetate.

The crystal are dissolved in 50% acetone bufferered with ammonium hydroxide prior to analysis by thin layer chromatography. The seeds are dried before grinding. The residual mass is preferably dissolved in 80% acetone. The supernatant layer is treated with 6N sulfuric acid.

Thin glass plates (20 X 20) coated (0.4 mm to 0.5 mm thick) with silica gel G are activated at 100°C, the solution of insulin applied, the plates developed in n-butanol, water, acetic acid (12:5:2), dried, the single spot corresponding to standard insulin visualized by spraying nin-hydrin (0.25%) in acetone, isolated along the silica gel G from unsprayed plates, extracted in 50% ethanol buffered with ammonium hydroxide, filtered, the filtrate dried and pure white needle-like crystals obtained.

The analysis is carried out, the isolated substance is hydrolyzed along with the standard insulin, applied on paper chromatograms separately, developed, yielding same amino acids except methionine being extra amino acids in the polypeptide-k totaling to 18 different amino acids, glutamine was present whereas it was absent in polypeptide-p.

The isolated substance and the standard insulin are hydrolyzed separately by 6N HCl for 20 hrs, dried, reconstituted in 50% ethanol, applied on Whatman No. 1 filter paper strips developed in n-butanol, acetic acid, water (60:320:20), strips developed sprayed with 0.25% nin-hydrin in acetone, same amino acids as of the standard hydrolyzate except methionine being extra in polypeptide-k along with glutamine, totalling to 18 different amino acids.

The analysis is carried, the seeds are extracted in hexane, acetone yielding a product which has the melting point (234°C), Gel electrophoretic pattern of the accompanying drawing the number of amino acids of the standard insulin except methionine being extra in polypeptide-k totalling to 18 different amino acids, glutamine was present in polypeptide-k.

THE FOLLOWING TYPICAL EXAMPLE IS GIVEN TO ILLUSTRATE THE INVENTION

#### EXAMPLE

The seed of Momordica Charantia L., (bitter gourd) were splitted, washed with water thoroughly, processed in hexane and acetone, dried and powdered and treated again in hexane and acetone and the residual mass was dissolved in 80% acetone. The pH was adjusted to 9.5 by adding ammonium hydroxide / organic buffer, the supernatant thus obtained was buffered to pH3 with 6N H<sub>2</sub>SO<sub>4</sub> for obtaining flocculent precipitate which were collected and crystallised with zinc acetate.

Thin glass plated (20 X 20 cm) coated (0.4 mm to 0.5 mm thick) with silica gel G (Kieselgel G nach Stahl; E. Merck) were activated at 100° C for half an hour. The solution containing the isolated substance was applied 1 cm above the edge of the plates were run in an organic solvent mixture of n- butanol, water and acidic acid (12:5:2). The developed plates were dried at room temperature and sprayed with 0.25% ninhydrin in acetone. The ninhydrin positive spots (R = 0.19) of the isolate corresponding to insulin were collected from about 200 unsprayed plates along with the silica gel G and extracted with 50% ethanol buffered with ammonium hydroxide. The extract was filtered and dried in vacuo. Pure colorless crystals thus obtained were weighed ( 3g/100 gram dry weight of seeds).

The melting point of the purified compound (232° - 235°C) as well as the standard insulin were determined. The melting point of the standard insulin was recorded as 233°C.

The standard sample of insulin as well as the isolated polypeptide-k were hydrolysed under reflux with 6N HCl for 20 hrs. separately. The hydrolyzates were filtered, dried, reconstituted separately in 50% ethanol and applied on strips

of Whatman No. 1 paper. The paper strips were run in an organic solvent mixture of n-butanol, water and acetic acid (60:20:20). The hydrolyzate of both the isolated and the standard insulin were also applied separately along with the known amino acids (hydroxylysine, methionine, hydroxyproline, tryptophan and glutamine). The various developed chromatograms were sprayed with 0.25% ninhydrin in acetone. The amino acids of the hydrolyzate of the standard coincided exactly with those of the hydrolyzate of the isolated compound except methionine an extra amino acid in isolated polypeptide-k. Hydroxylysine, hydroxy-proline and tryptophan were found to be absent from the hydrolyzate of the isolated polypeptide-k as well as of the standard hydrolyzate which gave an indication that the isolated polypeptide-k is nearly identical with that of the insulin except methionine being an extra amino acid and the total amino acids numbered to 18 different amino acids.

*Pnasifae Khanna*

Disc electrophoresis was carried out (10% SDS Biophore Gel, run in tris buffer, operating pH 6.1, 3% acetic acid in lower cell ; 90 V, mA 2.5 per tube; Bromophenol blue tracking dye). Samples of the crystallized isolate and bovine insulin containing dithiothreitol and EDTA, injected and run for 7 hr. Gel collected from the tubes were stained (0.05% coomassie Brilliant Blue R-250 in 7% aqueous acetic acid) and washed with 10% acetic acid. Electrophoretic pattern of both the isolate and the bovin insulin were nearly identical as shown in figure 1 of the accompanying drawings. Immuno assays of polypeptide-k did not show any cross reaction when tested with bovin insulin.

Sublingual administration of the isolate showed positive and highly effective hypoglycaemic activity. When five hundred diabetic patients were treated (Table 1) with polypeptide-k, no side effects of the drug were observed. Neuropathy, lethargicity, hypoglycaemia were not reported in these patients even when the drug was administered for a period of 2-4 years. At the same time sugar level in the blood came down appreciably in one month time. The results are shown in table 1.

TABLE – 1

Effect of polypeptide-k (Gourdin) on blood sugar level in patients with diabetes mellitus.

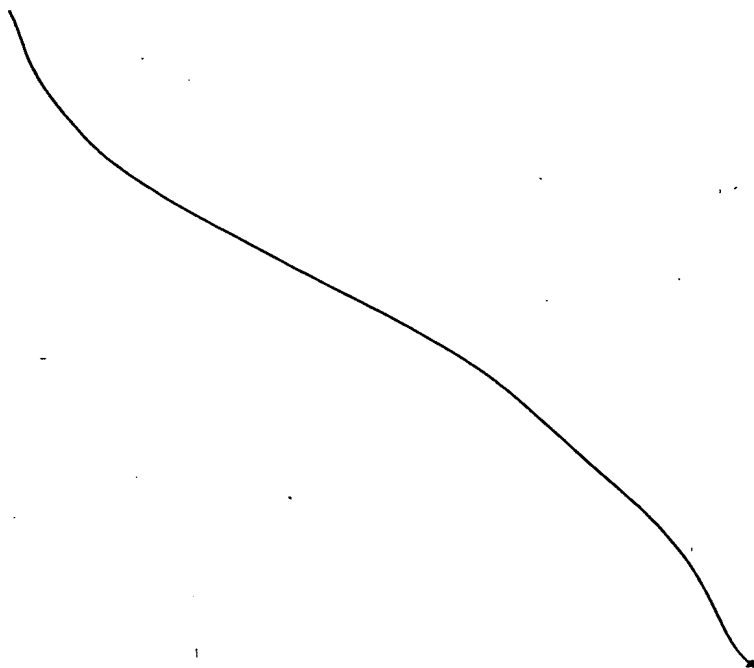
No. of Subjects	Diabetes Duration (Yrs.)	+ Range of blood sugar level mg/dl (post prantl)	* Gourdin effect Mean mg/dl fall in blood sugar level (after 2-7 days)
250	2-5	160-200	120-110
250	6-10	210-350	150-190
100	11-15	355-450	250-270
5	15-20	460-500	280

+ Most of the patients were taking drugs (Diaonil / Glyciphase / Glynase / DBI / Euglucon / Dimicron or combination of glyciphase and glynase.

\* Gradual fall in blood sugar level after one week to 40 days and then it came to normal. Continuation of polypeptide-k (Gourdin) intake varied from 6 months to 3 years. Four doses of polypeptide-k (Gourdin) before 10-15 minutes before each meal to be taken sublingually. In patients with blood sugar level from 355 or more diaonil in doses of 2 (1+1) or 1 ( $\frac{1}{2}$  +  $\frac{1}{2}$ ) was supplemented with gourdin doses (morning, evening). Diaonil was withdrawn completely after 15 days.

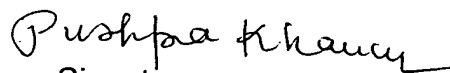
**THE FOLLOWING ARE THE ADVANTAGES:**

- 1 The polypeptide-k isolated from seeds by the process as claimed in earlier patent No. 176040 has been made more effective by improving upon the extraction procedure.
  2. The polypeptide-k was highly sublingually effective hypoglycemic drug.
  3. The cholestrol level including total cholestrol, HDL, LDL, VLDL and triglyceride go down to normal using this drug as an antidiabetic remedy.
  4. Symptoms as Leg pain, Lethargicity hypoglycaemia did not appear when more than 500 patients suffering for 2-4 years were treated with this drug.
- This invention as described in the example is merely illustrative in nature and not intended to restrict the scope of the invention.



## CLAIMS:

1. An Improved process for preparation of a sublingually highly effective hypoglycaemic novel protein (Polypeptide-K) from seeds of Momordica Charantia L. (bitter gourd) which comprises splitting the seeds of said Momordica Charantia, washing thoroughly the splitted seeds with water to remove contaminants, drying, powdering and treating the powder with hexane and acetone (2:1) to remove oil, drying the residual mass and dissolving the same in water and acetone, adjusting the pH to 9.5 by adding ammonium hydroxide/organic buffer, separating the supernatant layer from the mixture, treating the supernatant layer with sulfuric acid to adjust the pH to 3 to obtain the flocculent precipitate of polypeptide-k hydrolyzate, and crystallizing the said polypeptide -k with zinc acetate.
2. A process as claimed in claim 1 where in the seeds are dried before grinding.
3. A process as claimed in claim 1 & 2 wherein the residual mass is preferably dissolved in 80% of acetone.
4. A process as claimed in any of preceding claim wherein the supernatant layer is treated with ammonium hydroxide and then 6N Sulfuric acid resulting in precipitation.
5. An improved process of preparation of sublingually highly effective hypoglycaemic polypeptide-k substantially as herein before described in the reference to any of the examples.

  
Signature

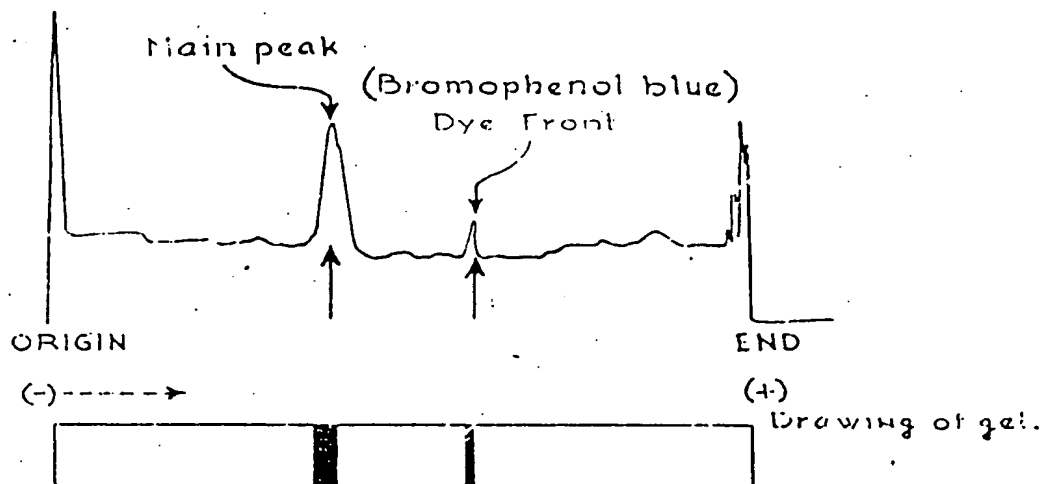
(Pushpa Khanna)

Dated : 17<sup>th</sup> January, 2002.

Applicant Name : Pushpa Khanna

No. of Sheets : 1

Sheet No. : 1



SCANNING OF THE POLYACRYLAMIDE  
GEL AFTER ELECTROPHORESIS ON  
CHROMOSCAN

Signature: *Pushpa Khanna*

Name : Pushpa Khanna

"Applicant"

Application No. : 560 DEL





GOVERNMENT OF INDIA  
MINISTRY OF COMMERCE & INDUSTRY,  
PATENT OFFICE, DELHI BRANCH,  
W - 5, WEST PATEL NAGAR,  
NEW DELHI - 110 008.

*I, the undersigned being an officer duly authorized in accordance with the provision of the Patent Act, 1970 hereby certify that annexed hereto is the true copy of the Application, Complete Specification and Drawing Sheets filed in connection with Application for Patent No.561/Del/99 dated 13<sup>th</sup> April 1999.*

*Witness my hand this 11<sup>th</sup> day of May 2004.*

A handwritten signature in black ink, appearing to read 'S.K. Pangasa', with a long horizontal flourish extending to the right.

**(S.K. PANGASA)**

*Assistant Controller of Patents & Designs*



0561 DEL 99

13 APR 1999

## FORM I

**THE PATENT ACT, 1970**  
**APPLICATION FOR PATENT WHEN THE TRUE AND FIRST INVENTORS**  
**ARE THE SOLE JOINT APPLICANTS (SEE SECTION)**

I, PROF. (DR.) MISS PUSHPA KHANNA (RETD.) E-14/7, VASANT VIHAR, NEW DELHI - 110057. INDIAN CITIZEN, hereby declare :

- (I) that I, Pushpa Khanna is in possession of an invention relates to a highly effective hypoglycaemic polypeptide-~~h~~
- (II) that I, Pushpa Khanna claims to be true and first inventor therefore;
- (III) that the specifications filled with this application is complete and any amended specification which may hereafter be filed in the behalf will be, true of the invention to which this application relates;
- (IV) that I, Pushpa Khanna believe that I, Pushpa Khanna is entitled to a patent for the said invention having regard to the provision of the Patent Act, 1970;
- (V) that to the best of my knowledge, information and belief the facts and matters stated herein are correct and there is no lawful ground of objection to the grant of patent to me on this application.

I, Pushpa Khanna request that a patent may be granted to me for the said invention and I, Pushpa Khanna request that all notices, requisitions and communications relating to this application may be sent to Dr. PUSHPA KHANNA, E-14/7, VASANT VIHAR, NEW DELHI - 110057.

Dated this      day of April, 1999

  
(signed) 1. Pushpa Khanna

To

The Controller of Patents  
The Patent Office  
Karol Bagh  
New Delhi



FORM 3A

0561 DEL 99


THE PATENTS ACT, 1970

13 APR 1999

Complete Specifications  
(See Section 10)

1. AN INVENTION RELATES TO ~~AN IMPROVED PROCESS FOR PREPARATION~~ OF HIGHLY EFFECTIVE HYPOGLYCAEMIC POLYPEPTIDE-~~FROM A PLANT SOURCE~~
2. PROF. (DR.) MISS PUSHPA KHANNA (RETD.) E-14/7, VASANT VIHAR, NEW DELHI - 110057. INDIAN CITIZEN

The following specifications particularly described and ascertains the nature of this invention and the manner in which it is to be performed :-



This invention relates to a highly effective hypoglycaemic polypeptide-p. This invention particularly relates to sublingually highly effective hypoglycaemic polypeptide-p <sup>from seeds of Momordica charantia L.</sup> and a process of the preparation thereof.

Insulin has hitherto been commercially or synthesised from the pancreas of animals and human insulin from E. Coll (Eli-Lilly, U.S.A.). So far there is no commercial extraction of insulin / polypeptide-p from plant source.

Isolation of Insulin from animal pancreas is open to objection due to the following reasons:

1. By killing 10,000 animals only one pound of pure insulin is obtained.
2. It is not being sublingually administered.
3. If the pancreas are infected by some diseases like Cancer etc. there is always a probability of its being carried (if it is a virus) along with the insulin.
4. Human Insulin can be synthesised from E. Coll, which is expensive.
5. Any amount of polypeptide-p can be synthesised from plant which is so easy to cultivate.

*P. Khan* An attempt was made to extract polypeptide-p <sup>seeds of Momordica charantia L.</sup> from plants by the present applicant. The method used was discussed in old Indian Patent specification No. 13656/45 which removed the above mentioned drawbacks. The fruits and cultures are separately extracted in ethanol and then mixed with cold ethanol and diethyl ether, needle-like crystals formed by adding zinc in traces after 18 hr. The fruits and cultures are separately crushed, homogenized in water, ethanol and concentrated sulfuric acid adjusting pH 1.5 - 2.0, filtered, filtrate.

This method had the following drawbacks:

1. The use of alcohol in the extraction procedure was not practical due to its unavailability in large amount and the impurities present in it.
2. The use of raw material as fruits and tissue culture creates problems in handling and uneconomic viability respecting and the yield was very poor.

In order to obviate the above drawbacks another process was developed for extraction a highly effective polypeptide-~~p~~ by using hexane alongwith diethylether which was claimed in Indian patent specific No. 176040. Although the process developed and disclosed in above referred patent specification resulted into good yield, improved purity and high efficacy of the drug by removal of oil and sapogenins and other contaminants therefrom. Yet it had some drawbacks :

1. The purification of polypeptide-p was cumbersome method due to the presence of interfering radicals as oil and sapogenins.
2. Use of diethylether in the extraction procedure was not practical due to its being highly inflammatory and involving high cost.
3. The presence of pesticides / insecticides / urea and other contaminants affected the purity of polypeptide-p.
4. The yield was not optimum.

Now I have developed an improved process for extracting a highly effective hypoglycaemic polypeptide-p and obviated the above drawbacks. In this process the splitted seeds are thoroughly washed with water many times to remove the last traces of pesticides / insecticides / urea and other contaminants.

The dried seeds are processed using hexane (food grade) along with acetone instead of either as used in the process described in earlier Patent No. 176040. The process has resulted into high yield, improved purity and high efficacy of drug by removal of undesired oils, flavonoids and sapogenins therefrom. The doses of resulted drug were administered sublingually to diabetic patients and showed excellent results in lowering the sugar level.

P. Khanu Accordingly the present invention provides a sublingually highly effective hypolycaemic polypeptide-~~k~~ from seeds of Momordica charantia L. (bitter gourd) by a process which comprises, splitting the seeds of Momordica charantia L., washing thoroughly the splitted seeds with water to remove contaminants if any, treating the splitted seeds with again solvents consisting hexane and acetone, grinding the said seeds to obtain powder, treating the said powder of seeds with hexane and acetone solvent, dissolving the residual mass in aqueous acetone, adjusting the pH upto 9.5 by adding ammonium hydroxide / organic buffer, separating the supernatant layer from the mixture, treating the supernatant layer with sulfuric acid by adjusting the pH upto 3 to obtain the flocculent precipitate of polypeptide-p, crystallizing the polypeptide-p.

The crystals are dissolved in 50% acetone buffered with ammonium hydroxide prior to analysis by thin layer chromatography. The seeds are dried before grinding. The residual mass is preferably dissolved in 80% acetone. The supernatant layer is treated with 6N sulfuric acid

Thin glass plated (20 X 20) coated (0.4 mm to 0.5 mm thick) with silica gel G are activated at 100 ° C, the solution of insulin applied, the plates developed in n-butanol, water, acetic acid (12:5:2), dried, the single spot corresponding to standard insulin visualized by spraying nin-hydrin (0.25%) in acetone, isolated along with silica gel G from unsprayed plates, extracted in 50% ethanol buffered with ammonium hydroxide, filtered, the filtrate dried and pure white needle - like crystals formed.

The analysis is carried out, the isolated substance is hydrolyzed along with the standard insulin, applied on paper chromatograms separately, developed, yielding same amino acids except methionine being an extra amino acid in the polypeptide-p.

The isolated substance and the standard insulin are hydrolyzed separately by 6 N HCl for 20 hours, dried, reconstituted in 50% ethanol, applied on Whatman No. 1 filter paper strips developed in n-butanol, acetic acid, water (60:320:20), strips developed sprayed with 0.25% nin-hydrin in acetone, amino acids same as of the standard hydrolyzate except methionine being extra in polypeptide-p.

The analysis is carried, the seeds are extracted in hexane, acetone yielding a product which has the same melting point (234° C), Gel electrophoretic pattern of the accompanying drawing and number of amino acids of the standard insulin except methionine being extra in polypeptide-p.

THE FOLLOWING TYPICAL EXAMPLE IS GIVEN TO ILLUSTRATE THE INVENTION

### EXAMPLE 1

The seed of *Momordica Charantia* L., (bitter gourd) were splitted, washed with water thoroughly, processed in hexane and acetone, dried and powdered and treated again in hexane and acetone and the residual mass was dissolved in 80 % acetone. The pH was adjusted to 9.5 by adding ammonium hydroxide / organic buffer, the supernatant thus obtained was buffered with 6N  $H_2SO_4$  for obtaining in flocculent precipitate which were collected and crystallised with zinc acetate.

Thin glass plated (20 X 20 cm) coated (0.4 mm to 0.5 mm thick) with silica gel G (Kieselgel G nach Stahl; E. Merck) were activated at 100° C for half an hour. The solution containing the isolated substance was applied 1 cm above the edge of the plates were run in an organic solvent mixture of n-butanol, water and acidic acid (12:5:2). The developed plates were dried at room temperature and sprayed with 0.25% ninhydrin in acetone. The ninhydrin positive spots ( $R_f = 0.19$ ) of the isolate corresponding to insulin were collected from about 200 unsprayed plates along with the silica gel G and extracted with 50% ethanol buffered with ammonium hydroxide. The extract was filtered and dried in vacuo. Pure colorless crystals thus obtained were weighed (3 g/100 gram dry weight of seeds).

The melting point of the purified compound (232° - 235° C) as well as the mm (234° C) were determined). The melting point of the standard insulin was recorded as 233° C.

The standard sample of insulin as well as the isolated polypeptide-p were hydrolyzed under reflux with 6N HCl for 20 hrs. separately. The hydrolyzates were filtered, dried, reconstituted separately in 50% ethanol and applied on strips of Whatman No. 1 paper. The paper strips were run in an organic solvent mixture of n-butanol, water and acetic acid (60:20:20). The

hydrolyzates of both the isolated and the standard insulin were also applied separately along with the known amino acids (hydroxylysine, methionine, hydroxyproline and tryptophan). The various developed chromatograms were sprayed with 0.25% ninhydrin in acetone. The amino acids of the hydrolyzate of the standard coincided exactly with those of the hydrolyzate of the isolated compound except methionine being an extra amino acid in isolated polypeptide-p. Hydroxylysine, hydroxy-proline and tryptophan were found to be absent from the hydrolyzate of the isolated polypeptide-p as well as of the standard hydrolyzate which gave an indication that the isolated polypeptide-p is nearly identical with that of the insulin.

Disc electrophoresis was carried out (10% SDS Biophore Gel, run in tris buffer, operating pH 6.1, 3% acetic acid in lower cell ; 90 V, mA 2.5 per tube; Bromophenol blue tracking dye). Samples of the crystallized isolate and bovine containing dithiothreitol and EDTA, injected and run for 7 hr. Gel collected from the tubes were stained (0.05% coomassie Brilliant Blue R-250 in 7% aqueous acetic acid) and washed with 10% acetic acid. Electrophoretic pattern of both the isolate and the bovine insulin were nearly identical as shown in figure 1 of the accompanying drawings. Immuno assays of polypeptide-p did not show any cross reaction when tested with bovine insulin.

Sublingual administration of the isolate showed positive and highly effective hypoglycaemic activity. When five hundred diabetic patients were treated (Table 1) no side effects of the drug were observed. Neuropathy, lethargy, hypoglycaemia were not reported in these patients even when the drug was administered for a period of 2-4 years. At the same time sugar level in the blood came down appreciably in one month time. The results are shown in table 1.

TABLE - I

Effect of Gourdin (polypeptide-p) on blood sugar level in patients with diabetes mellitus.

No. of Subjects	Diabetes duration (yrs.)	* Range of blood sugar level mg/dl (post prandl)	* Gourdin effect Mean mg/dl fall in blood sugar level (after 2-7 days)
250	2-5	160-200	120-110
250	6-10	210-350	150-190
100	11-15	355-450	250-270
5	15-20	460-500	282

+ Most of the patients were taking drugs (Diaonilic/Glyciphage/Glynase/DBI/Euglucon/Dimicron or combination of glyciphage and glynase).

\* Gradual fall in blood sugar level after one week to 40 days and then it came to normal. Continuation of Gourdin intake varied from 6 months to 3 years. Four doses of Gourdin before 10-15 minutes before each meal to be taken sublingually. In patients with blood sugar level from 355 or more diaonil in doses of 2 (1+1) or 1 (1/2+1/2) was supplemented with gourdin doses (morning, evening). Diaonil was withdrawn completely after 15 days.

**THE FOLLOWING ARE THE ADVANTAGES:**

1. The polypeptide-p isolated by the process as claimed in earlier patent No. 176040 has been made more effective by improving upon the extraction procedure.
2. The polypeptide-p was highly sublingually effective hypoglycemic drug.
3. The cholestrol level including total cholestrol, HDL, LDL, VLDL and triglycesied go down to normal using this drug as an autidiabetic remedy.
4. Symptoms as Leg pain, Lethargicity hypolycarinia did not appear when more than 500 patients of 2-4 years with this drug was given. This invention as described in the example is merely illustrative in nature and not intended to restrict the scope of the invention.

## CLAIM

- P. Khanna
1. A sublingually highly effective hypoglycaemic polypeptide-~~h~~ from seeds of Memordica Charantia L., (bitter gourd) ~~by~~ prepared by a process which comprises, splitting the seeds of Memordica Charantia L. (bitter gourd), washing thoroughly the splitted seeds with water to remove contaminants, treating the splitted seeds with solvent consisting of hexane and acetone, grinding the said seeds to obtain powder, treating the said powder of seeds with, hexane and acetone solvents, dissolving the residual mass in aqueous acetone, adjusting the pH upto 9.5 by adding ammonium hydroxide / organic buffer, separating the supernatant layer from the mixture, treating the supernatant layer with sulfuric acid by adjusting the pH upto 3 to obtain the flocculent precipitate of polypeptide-p, crystallizing the said polypeptide-p with zinc acetate.
  2. A hypoglycaemic polypeptide-~~h~~ as claimed in claim 1 / where in the seeds are dried before grinding.
  3. A hypoglycaemic polypeptide-~~h~~ as claimed in claim 1 & 2 wherein the residual mass is preferably dissolved in 80% of acetone.
  4. A hypoglycaemic polypeptide-~~h~~ as claimed in any of preceding claim wherein the supernatant layer is treated with 6N Sulfuric acid.
  5. A sublingually highly effective hypoglycaemic polypeptide-~~h~~ substantially as here in before described with reference to any of the forgoing examples.

Dated 14<sup>th</sup>. Day of April, 1999

Signature

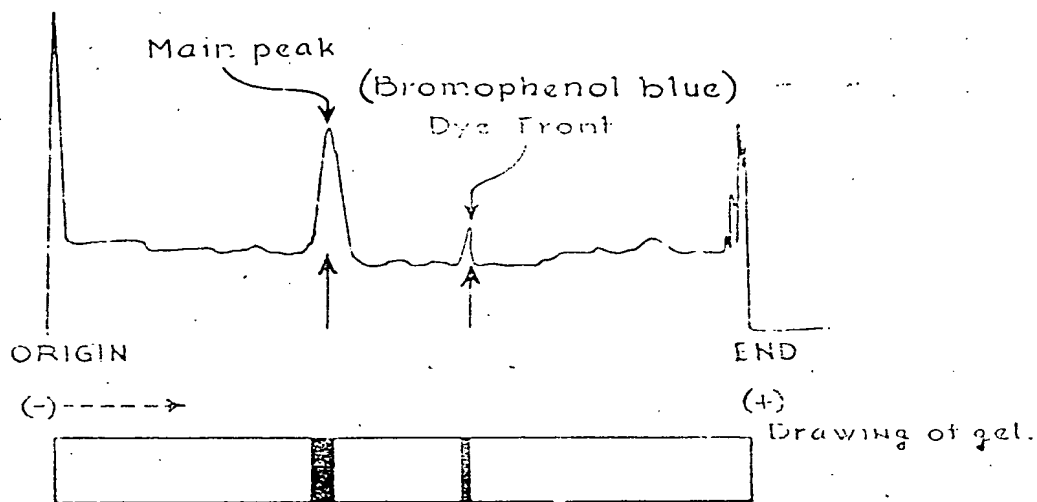
*Pushpa Khanna*

(Pushpa Khanna)

Applicant Name : Pushpa Khanna

No. of Sheets : 1

Sheet No. : 1



SCANNING OF THE POLYACRYLAMIDE  
GEL AFTER ELECTROPHORESIS ON  
CHROMOSCAN

56/10/29  
13/4/29

Signature: Pushpa Khanna

Name : Pushpa Khanna

"Applicant"

